

## Absorption of different lead compounds

D. BARLTROP  
M.D., F.R.C.P.

F. MECK  
A.A.I.M.T., A.R.M.I.T.

*Paediatric Unit, St Mary's Hospital Medical School, London*

### Summary

A rapid method for the determination of relative absorption of dietary lead by rats is described. The influence of age, weight and dose rate has been determined and using standard conditions the tissue lead content of blood, kidney and femur are significantly correlated with each other and are a function of ingested lead. Eight lead compounds were evaluated using this technique and the findings related to lead acetate as a reference compound. Of the inorganic preparations studied, lead carbonate (basic) and metallic lead showed a twelve-fold difference in absorption, with the remaining compounds giving intermediate values. The absorption of lead from four organic compounds was determined from diets containing 7.5% corn oil added to the standard diet. Lead tallate was absorbed to the same degree as lead acetate, but lesser absorptions resulted from lead octoate, naphthenate and alsynate. The addition of corn oil to a final concentration of 7.5% of the diet enhanced the absorption of lead acetate.

### Introduction

In the majority of lead poisoning cases, lead enters the body orally. This contaminating lead exists in the environment in many different chemical forms, lead sulphide or lead chloride in the mining industry, lead oxide in soil adjacent to smelters, lead carbonate, lead chromate and other lead salts used as pigments in older paints. Today the paint industry uses lead octoate, naphthenate and tallate as driers, and in the home cooking utensils and badly glazed pottery can cause lead contamination of food. It has been thought that not all forms of lead are absorbed at the same rate, but information available on relative toxicities is conflicting and poorly documented (Fairhall and Sayers, 1940; Allcroft, 1950; Kaurhausen, 1972). Some evidence from the mining industry suggests that galena (lead sulphide) represents a minimal hazard to adults, whereas lead chloride is relatively toxic. The absorption of lead from soils by plants has been related to the solubility in water of the lead compounds concerned (Maclean, Halstead and Finn, 1969) and it has been suggested that compounds which are soluble or able to be converted to soluble

compounds in the gut are most readily absorbed (Kaurhausen, 1972). Increasing attention to the possibility of differential toxicity has been given in connection with paints and surface coating materials, although one limited study has suggested that lead additives to polymers are poorly absorbed (Gage and Litchfield, 1968). In order to investigate the relative absorptions of a wide range of organic and inorganic lead compounds, a rapid and reliable screening method was devised. Preliminary studies were undertaken to validate the selected methodology and to select standard conditions for dose rate, animal age and body weight. It has been demonstrated that lead absorption in the rat decreased from 90% to 16% of the ingested dose between the twentieth and thirty-second day of life (Forbes and Reina, 1972). This work, however, used a carrier-free lead isotope and the results might not apply where large doses of oral lead are involved. This paper reports the findings from preliminary studies and the initial results for a series of lead compounds.

### Methods and materials

The rat was selected as the experimental animal as the absorption of lead in this species has been studied extensively in this department and by other workers. For each lead compound, eight male Wistar rats of 90-110 g body weight, and aged 30-32 days were used. Two animals were killed on the first day of the trial to confirm that they had not previously been exposed to lead. The remainder were used as test animals and housed in individual stainless steel and polythene cages. Lead acetate was chosen as a standard reference compound of known high lead absorption and was administered at intervals throughout the trials to test the reproducibility of the method. Some of the organic lead compounds used in the paint industry are oily viscous liquids and required solution in corn oil before incorporation into the diet. For these compounds lead acetate in corn oil was used as a reference standard. Controls were also run at intervals during the trials in which rats were fed a diet containing no added lead. Oxoid powdered 41B (modified) diet containing 2.0 µg lead/g was prepared to contain 0.075% lead as the

appropriate compound, 5% molasses and then baked to form a firm stick diet. This diet was analysed for lead and found to be homogeneous. Six test animals were supplied with a weighed portion (33–35 g) of lead-containing diet, any remaining after 48 hr was re-weighed and the amount of lead ingested calculated by difference. Tap water containing less than 10 µg/l was provided *ad libitum*. The animals were re-weighed, killed by ether anaesthesia and dissected immediately. Approximately 2 ml of blood were taken from the vena cava with a disposable syringe and needle, and collected into lead-free heparinized tubes. Kidneys and the right femur were removed. Blood and kidney specimens were wet ashed and taken to dryness using a sulphuric acid, nitric acid and perchloric acid digestion mixture on a sandbath at 350°C. The femurs were dry ashed in a muffle furnace for successive periods of 20 hr at 450°C and 600°C. The ash was weighed and dissolved in 0.5 ml of concentration hydrochloric acid (Foodstuffs Analysis Grade) and diluted to 10 ml with distilled deionized water. An aliquot was evaporated to dryness at 85–90°C. In each case the residues were dissolved in acid ammonium citrate buffer and analysed by an automated dithizone method (Browett and Moss, 1965). Standard statistical techniques were used in interpreting the data, namely Student's *t*-test and the Mann-Whitney non-parametric test for the significance of differences between the lead content of given tissues, and correlation coefficients for the relation between lead contents of different tissues.

## Findings

### Dose studies

The selection of the lead content for the diets was based on experiments designed to establish a dose response curve. Forty-two 32-day-old male Wistar rats weighing 90–110 g were allocated at random into seven equal groups, and fed varying doses of lead acetate in the range 0.04–1.0% lead for 48 hr. Tissue lead concentrations increased with dose and the response for kidney lead concentrations is shown graphically in Fig. 1. Blood, kidney and femur lead contents were all found to be well correlated (Table 1 and Fig. 2). In a further study lead naphthenate dissolved in corn oil was used and data were obtained of a diet lead content up to 0.2%, above which the rats found the diet to be unpalatable. Much greater tissue concentrations were observed with lead naphthenate than with lead acetate but the curves were of a similar form with an initial rapid increase falling off slightly after a dose of 0.01% lead in the diet. The value 0.075% lead was selected for the trials since it lay on the most sensitive sections of the dose response curves and was great enough to produce significant tissue lead concentrations after 48 hr.

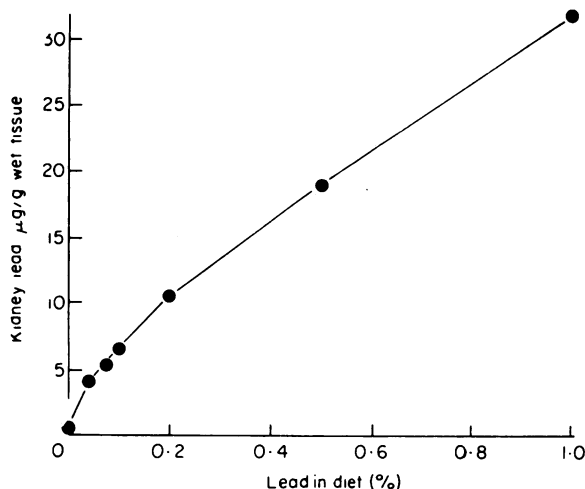


FIG. 1. Mean kidney lead concentrations for groups of six animals after 48 hr on diets of varying added lead content.

TABLE 1. Correlation between tissue lead contents

Tissues	r	d.f.	P
Blood v. kidney	0.92	40	<0.001
Blood v. femur	0.94	40	<0.001
Kidney v. femur	0.97	40	<0.001

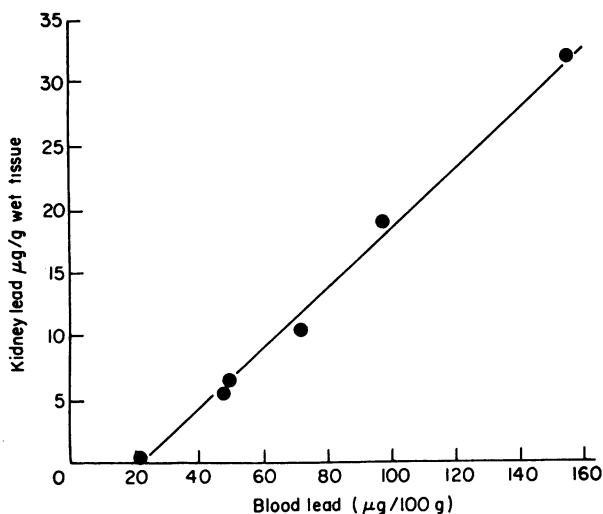


FIG. 2. Correlation between blood and kidney lead concentrations showing regression line and mean values ( $r = 0.92$ ; d.f. = 40;  $P < 0.001$ ).

Although greater tissue lead concentrations could have been attained with greater doses, the value was kept as low as possible, bearing in mind the small doses involved in childhood toxicity.

### Age study

Following the report of large changes in lead absorption in young rats (Forbes and Reina, 1972), an experiment was designed to investigate the effect of age on lead absorption in rats under our experimental conditions. Forty-two rats were allocated at random into seven groups and fed with a control diet. At intervals during the age range 21–42 days, the control diet was withdrawn from successive groups and a diet containing 0.075% lead as lead acetate substituted for 48 hr. Kidney lead concentration, as a function of the ingested dose, decreased from  $10.82\% \times 10^{-2}\%$  to  $2.70 \times 10^{-2}\%$  between 21 and 42 days of life (Fig. 3). Although this effect was small, rats aged 30–32 days were selected for the lead compound trials to ensure minimum variation from this source.

A further study was made to investigate any influence that body weight might exert on lead absorption at a given age. Although the findings

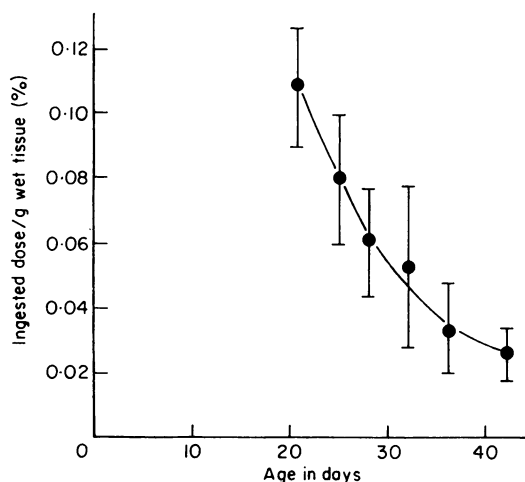


FIG. 3. Percentage ingested dose of lead per gram of wet weight kidney as a function of age for groups of six animals.

were considered inconclusive, to ensure minimum variation, animals of body weight 90–110 g were selected.

### Compound studies

In addition to controls and the reference compound, lead acetate, twelve lead compounds were tested (Table 2). For each compound, blood, kidneys and right femurs were analysed for lead. Lead acetate without added corn oil gave a mean kidney lead content of  $11.4 \mu\text{g}/\text{kidney}$ . Of all the other solid compounds, only basic lead carbonate had a greater kidney lead content than the standard reference compound. Metallic lead was considerably less, lead sulphide and lead chromate were intermediate but still significantly different from lead acetate,  $P < 0.001$  (Fig. 4). Lead acetate with 7.5% corn oil in the diet resulted in a kidney lead content of  $20 \mu\text{g}/\text{kidney}$ . Lead tallate was not significantly different from this reference whereas lead octoate and lead naphthenate were significantly less,  $P < 0.02$  and  $P < 0.01$  respectively (Fig. 5). The kidney lead contents were calculated as percentages of the relevant lead acetate values (Table 3). Every compound tested was shown to differ significantly from the controls ( $P < 0.001$ ).

### Discussion

The technique described was found to be a rapid and reliable method for the evaluation of lead absorption from different lead compounds in the environment. Although animal age and weight variables were rigidly controlled in order to minimize variation from these sources, it is likely that they had a relatively small effect. Distinction must be made between the findings reported by other workers (Forbes and Reina, 1972; Kostial, Simonović and Pisonić, 1971) using carrier-free isotopes of lead, and the present work in which an appreciable concentration of stable lead was used. Although the dosages employed were relatively great compared with those likely to be encountered in childhood lead

TABLE 2. Lead additives to animal diets

Lead (metallic)	Pb	Solid. Particle size 180–250 $\mu$ .
Lead oxide	PbO	
Lead sulphide	PbS	
Lead chromate	PbCrO <sub>4</sub>	Solids. Particle size < 50 $\mu$ .
Lead molybdate	PbMoO <sub>4</sub>	
Lead carbonate (basic)	2PbCO <sub>3</sub> , Pb(OH) <sub>2</sub>	
Lead chloride	PbCl <sub>2</sub>	
Lead stearate	Pb (C <sub>18</sub> H <sub>35</sub> O <sub>2</sub> ) <sub>2</sub>	Oily viscous liquids.
Lead octoate	Pb (C <sub>8</sub> H <sub>15</sub> O <sub>2</sub> ) <sub>2</sub>	
Lead tallate	24% lead	
Lead alsynate	24% lead	
Lead naphthenate	24% lead	

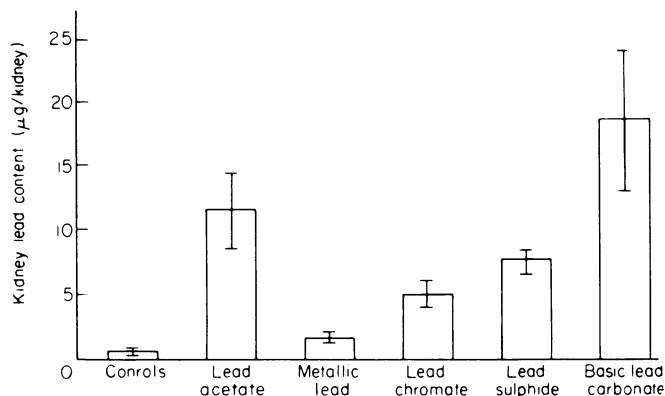


FIG. 4. Histogram showing the relative kidney lead contents for inorganic lead compounds.  $\pm 2$  s.d.

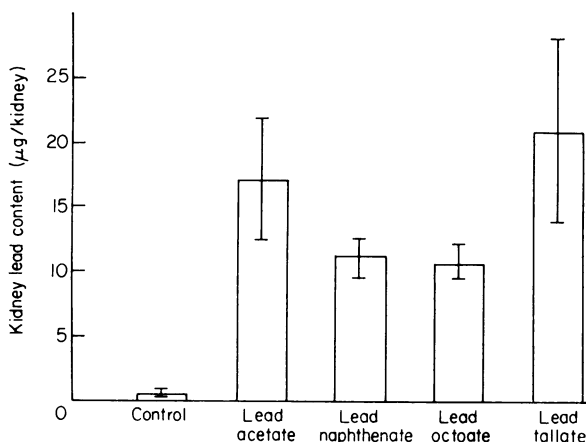


FIG. 5. Histogram showing the kidney lead content for controls, lead acetate and three organic lead compounds, incorporated into the diet with 7.5% corn oil.  $\pm 2$  s.d.

TABLE 3. Absorption of lead additives relative to lead acetate

Lead compound	Percent absorption
Control (no lead)	4
Metallic lead	
(particle size 180–250 $\mu$ )	14
Lead chromate	44
Lead octoate	62
Lead naphthenate	64
Lead sulphide	67
Lead tallate	121
Lead carbonate (basic)	164

poisoning, it is nevertheless argued that the findings are more realistic than those derived from carrier-free studies. Work in this department using identical doses of stable lead labelled with  $^{203}\text{Pb}$  has shown

that the rat absorbs only 1% of the ingested dose compared with 53% absorption that has been reported for children (Barltrop and Khoo, 1974; Alexander, Delves and Clayton, 1972). The tissue data derived from rats given lead compounds were well correlated, notably between the values obtained for blood, kidney, and bone lead content. This finding was unexpected and perhaps reflects the short-term nature of these studies.

There were significant differences in the lead absorbed from different lead compounds, as much as a twelve-fold difference being seen between metallic lead and basic lead carbonate. The high absorption from basic lead carbonate may reflect the greater solubility of this compound in gastric juice, but it is not possible to relate the observed differences in the other compounds to their solubility in biological fluids. Although recent legislation in the U.K. concerning pencils and graphic materials is based on the content of 'soluble lead' (the amount dissolved in hydrochloric acid under standard conditions), it is doubtful whether this reflects the situation concerning the lower part of the gut from which absorption occurs.

An important finding, substantiating other work in this department (Barltrop and Khoo, 1974), was that an increase in dietary fat increased the lead absorbed from a given lead compound. With lead acetate, the mean kidney lead content was raised from 11.4  $\mu\text{g}$  to 20  $\mu\text{g}$  with the addition of 7.5% corn oil to that diet.

These data suggest that it is not possible to devise a single relevant standard for the lead content of surface-coating materials and the various environmental situations in which lead contamination occurs. Reference should be made to the chemical form of the lead involved and perhaps to dietary factors relating to each situation.

### Acknowledgments

The project upon which this publication is based was performed pursuant to Contract No. HSM 99-73-28, the Center for Disease Control, Department of Health, Education and Welfare, United States of America.

Samples of lead compounds were kindly supplied by Associated Lead Manufacturers Ltd, Lankro Chemicals Ltd, Mooney Chemicals Ltd and Nuodex Ltd.

### References

- ALEXANDER, F.W., DELVES, H.T. & CLAYTON, B.E. (1972) The uptake and excretion by children of lead and other contaminants. *Proceedings. International Symposium on Environmental Health Aspects of Lead*, Amsterdam, p. 319.
- ALLCROFT, R. (1950) Lead as a nutritional hazard to farm animals. *Journal of Comparative Pathology and Therapeutics*, **60**, 190.
- BARLTROP, D. & KHOO, H.E. (1975) The influence of nutritional factors on lead absorption. *Postgraduate Medical Journal*, **51**, 795.
- BROWETT, B.V. & MOSS, R. (1965) Manual and semi-automated methods for the determination of the lead content of urine. *Analyst*, London, **90**, 715.
- FAIRHALL, L.T. & SAYERS, R.R. (1940) The relative toxicity of lead and some of its common compounds. *Public Health Bulletin* (U.S.) No. **253**.
- FORBES, G.B. & REINA, J.C. (1972) Effects of age on gastrointestinal absorption. *Journal of Nutrition*, **102**, 647.
- GAGE, J.C. & LITCHFIELD, M.H. (1968) The migration of lead polymers in the rat gastrointestinal tract. *Food and Cosmetics Toxicology*, **6**, 329.
- KAURHAUSEN, L.R. (1972) Intestinal lead absorption. *International Symposium on Environmental Health Aspects of Lead*, Amsterdam, p. 427.
- KOSTIAL, K., SIMONVIĆ, I. & PISONIĆ, M. (1971) Lead absorption from the intestine of newborn rats. *Nature*, London, **233**, 564.
- MACLEAN, A.J., HALSTEAD, R.L. & FINN, B.J. (1969) Extractability of added lead in soils and its concentration in plants. *Canadian Journal of Soil Science*, **49**, 327.